

# Zinc status and its association with the health of adolescents: a review of studies in India

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**Background:** Zinc is important in adolescence because of its role in growth and sexual maturation. Adolescents from developing countries such as India may be at high risk of zinc deficiency because of unwholesome food habits and poor bioavailability of zinc from plant-based diets.

**Objectives:** (1) to study zinc status and its association with profile of other micronutrients, (2) to construct a simple tool in the form of Adolescent Micronutrient Quality Index (AMQI) to assess quality of diets of the girls and (3) to examine the effect of zinc supplement on health of adolescent girls.

Methods: Girls (10–16 years) from two secondary schools of Pune, Maharashtra state, in Western India were enrolled in a cross-sectional study (n = 630). Data were collected on dietary intake, cognitive performance, taste acuity, haemoglobin, erythrocyte zinc and plasma levels of zinc, vitamin C, β-carotene and retinol. AMQI was developed using age–sex-specific Indian dietary guidelines and healthy foods and habits described in the recent US dietary guidelines. Zinc-rich recipes were developed considering habitual diets of the girls and vegetarian sources of zinc. An intervention trial (n = 180) was conducted to assess the effect of zinc-rich dietary supplements and ayurvedic zinc (Jasad) supplementation.

**Results:** Prevalence of micronutrient deficiencies was high in these girls. Poor cognitive performance was seen in half of the girls, and salt taste perception was affected in 45%. AMQI was correlated with nutrient intakes and blood micronutrient levels (p < 0.01), indicating the potential of AMQI to measure micronutrient quality of diets of adolescent girls. Results of the intervention trial indicated that supplementation of zinc-rich recipes vis-a-vis ayurvedic Jasad zinc has the potential to improve plasma zinc status, cognitive performance and taste acuity in adolescent girls.

**Conclusion:** Review of the studies on Indian adolescent girls demonstrates the necessity of adopting zinc and micronutrient-rich diets for positive health building in adolescents.

Keywords: zinc; micronutrients; India; cognitive function; taste acuity; supplementation; diet quality index

This article has been commented on by Vaman Khadilkar and Anuradha Khadilkar. Please follow this link http://www.globalhealthaction.net/index.php/gha/article/view/15366 to read their commentary.

Received: 4 June 2011; Revised: 11 March 2012; Accepted: 17 March 2012; Published: 12 April 2012

he world's adolescent population – 1,200 million persons 10–19 years of age, comprising about 19% of the total population – is considered to be a nutritionally vulnerable segment of the population. Adolescents face a series of serious nutritional challenges, such as energy-protein malnutrition and micronutrient deficiencies. Due to increasing nutritional demands for rapid

growth and inappropriate eating habits, prevalence of malnutrition is observed to be high in adolescents, which affects their growth and development. While the role of energy and proteins on physical growth is well established, recent studies have stressed the importance of micronutrients in enhancing full growth potential. However, 60–80% of adolescents globally suffer from micronutrient

deficiencies (1–5). These deficiencies can have long-term consequences such as impaired behavioural and brain development, delayed sexual maturation, loss of final adult height and osteoporosis in adolescents (6–8). Therefore, studying micronutrient status during adolescence has become an important area of concern as their biological functions in prevention of disease have been identified.

Amongst micronutrients, zinc is in the forefront due to the pervasive nature of zinc-dependent enzymes in metabolic processes; its vital role in several body functions such as vision, taste perception, cognition, cell reproduction, growth, immunity; and beneficial effect of zinc supplementation in many disease states. Zinc deficiency is a health problem in many communities, especially among adolescents because of their pubertal growth spurt. Nearly half of the world's population is at risk due to inadequate zinc intake, suggesting that public health programmes are urgently needed to reduce zinc deficiency (9).

To understand the nature and magnitude of the nutritional deficiencies, it is essential to investigate the dietary intakes and lifestyle factors of adolescents and examine their health risks. Changes in lifestyle may affect eating habits and food choices of adolescents. Eating healthy foods is an important part of a healthy lifestyle, and healthy nutritional practices need to be inculcated during child-hood and early adolescence. However, international research has reported unwholesome food habits in adolescents, such as consumption of soft drinks, fried foods and non-inclusion of fruits and vegetables in the diet (10, 11), leading to nutritional inadequacy, and particularly micronutrient deficiencies, in adolescents (2, 3, 12).

In Indian adolescents, inadequate intake of several micronutrients such as iron, vitamin A and C are widespread (13, 14, 15) because staple diets are predominantly cereal based, and intakes of meat, fish, dairy products, fruits and vegetables are low (4, 5, 16, 17). Further, cereal-based diets contain high amounts of phytate, which reduce the bioavailability of minerals (18), implying a higher risk of iron and zinc deficiencies in Indian adolescents. In our earlier study on married adolescent girls (15–19 years), it was observed that mean intakes of micronutrients including zinc were 40–75% less than the recommended dietary intakes (RDI) for Indian adolescent girls (19). However, there is no systematically collected information on prevalence of zinc deficiency across the country in India (20). Nevertheless, the predictions based on dietary patterns indicate that mild or moderate zinc deficiency could be widespread in Indian adolescents (21). Moreover, among Indian adolescents, girls are at more risk due to low social status (22), disparities in household work patterns, lack of education, early marriage, which determine the quality of diet for adolescent girls.

To ensure micronutrient quality of diets, dietary guidelines emphasise a need for including whole grains and a variety of vegetables and fruits in diets (23, 24). However, to evaluate diets for their nutritional adequacy there is a need to translate these guidelines into a simple measuring tool. Diet quality index or scores are tools used as a measure of diet quality, which assess compliance with prevailing dietary guidelines. For assessing nutrient adequacy of diet, various factors are considered, such as food variety, ratio of actual nutrient intake to recommended intake and so on (25–27). Some studies have reported measures of fruit and vegetable intakes in adolescents, which will be indirectly helpful in assessing the micronutrient quality of their diets (28, 29). However, none of these available indices gave due attention to food practices and consumption of type of foods, which promote bioavailability of micronutrients; also, neither of these focus on micronutrient consumption per se. Food habits such as consumption of tea with meal adversely affects mineral bioavailability due to presence of tannins (18, 30), whereas food processes such as sprouting and fermentation are known to degrade phytates, thereby enhancing the bioavailability of minerals (31, 32). Therefore, such practices must be considered while formulating the micronutrient quality index for vegetarians. In view of the increasing prevalence of vegetarianism (33), which is particularly high in India, it is imperative to create an index for evaluating micronutrient deficiency risk for vegetarians in general and for adolescent girls in particular.

To overcome the public health problem of nutritional deficiencies, food-based approaches such as dietary diversification in the form of locally available and culturally accepted foods at household level have been suggested (2, 34, 35). Enhancement of micronutrient density of habitual diets may be a more sustainable, economical, feasible and culturally acceptable method and can be used to alleviate several micronutrient deficiencies simultaneously without risk of antagonistic interactions (36). Most of the studies using supplementation strategies to alleviate nutrient deficiencies, mainly iron and zinc, have opted for enriching beverages, salts, or foods with multiple micronutrient combinations (17, 37, 38). Studies reporting dietary supplementation of zinc are few and have used animal foods to enhance zinc absorption (37, 39). Dietary intervention in rural Kenyan schoolchildren in the form of school snacks has been found to be beneficial in reducing micronutrient deficiencies, especially those of iron and zinc (37). Considering dietary habits of vegetarian adolescents, there is a need to develop sustainable dietary intervention using zinc-rich vegetarian food sources and adopting suitable cooking methods to improve the bio-available zinc intake of adolescents.

The effect of zinc supplementation can be measured using functional indices of zinc as an impact indicator (40,

41). Two of the functional indicators of zinc, namely taste acuity and cognitive testing, have been used to assess zinc status in childhood (40, 42). The exact mechanisms are not clear but it appears that zinc is essential for neurogenesis, neuronal migration, synaptogenesis, and its deficiency could interfere with neurotransmission and subsequent neuropsychological behaviour (43). Supplementation of zinc in US school children showed significant improvement in cognitive performance (44). However, a study carried out in China has reported supplementation of zinc with micronutrients was more effective than zinc alone in enhancement of neuropsychological performance and growth in children (45). In school children, the data are controversial, but evidence of improved neuropsychological function has been associated with zinc supplementation (43). In particular, the effect on cognitive performance in response to improved zinc nutrition is critical, given that zinc deficiency is common in both developing and developed countries (46).

The sense of taste is mediated through the salivary zinc-dependent polypeptide, gustin. Low salivary zinc concentration leads to a reduction of taste and reduced appetite (47). In young women, it has been reported that the ability to correctly discriminate salt concentrations shows a significant positive correlation with plasma zinc (48). In a cross-sectional study, children with low hair zinc levels were found to have higher median recognition threshold for salt than those with normal hair zinc levels (40). Such studies need to be done in adolescents.

Overall, the literature review suggests that: (a) micronutrient deficiencies are widespread in adolescents all over the world. Yet, information on micronutrient status. in particular of zinc status in Indian adolescents is lacking; (b) inadequate dietary intake of absorbable zinc is the primary cause of zinc deficiency, and dietary strategies are needed to improve the bio-available zinc intake of adolescents, especially that of girls; (c) a simple tool to assess the micronutrient quality of the diets of adolescents is required, which will help in identifying high-risk adolescents; and (d) intervention studies have confirmed the critical importance of adequate zinc nutrition to improve cognitive performance in children. However, little is known about the magnitude of effects of zinc supplementation on cognitive performance in adolescents.

# Rationale and significance of the study

Vitamin and mineral deficiencies adversely affect a third of the world's population (49), and it is estimated that one in five people worldwide lack sufficient dietary zinc (41). Adolescent girls may be more prone to zinc deficiency because physiological requirements for zinc peak during adolescence at the time of the pubertal growth spurt, which generally occurs in girls between 10 and 15 years (50). Especially, adolescents from developing countries

such as India are at high risk because of low zinc intakes and poor bioavailability of zinc from vegetarian diets, which leads to low zinc status. In adolescents, a wide range of disturbances occur due to zinc deficiency, including impaired growth, defects in the immune system, dermatitis, diarrhoea, delayed sexual and bone maturation, impaired taste acuity and behavioural changes (41). Therefore, more research is required to assess zinc status of Indian adolescent girls.

Simple measures such as diet quality index are used to assess adherence to dietary guidelines. To identify micronutrient deficiencies in adolescents, in particular that of zinc, a simple tool needs to be devised, which can judge the micronutrient quality of diet. Such an index would be of vital importance for identifying at-risk individuals and targeting the intervention programmes.

Guidelines by WHO have recommended food-based approaches to resolve micronutrient deficiencies (49). Moreover, zinc-rich dietary supplement has a potential to revert bad health effects of low zinc status. Therefore, increasing consumption of foods with a high content of absorbable zinc can become a long-term sustainable strategy to overcome zinc deficiency. Considering the enhancers and inhibitors in vegetarian diets (18), low-cost food recipes need to be devised to provide high bioavailable zinc.

Studies have documented positive effects of zinc supplements in infants and children for morbidity and growth (51). However, research on the beneficial effects of improved zinc nutrition for behaviour and cognitive function in school-age children is critically needed because zinc deficiency continues to be a modern health concern in both developing and developed countries (46, 52).

#### Study objectives

The present research aimed to study zinc status and its association with profile of other micronutrients, and to examine the effect of zinc supplementation on functional indices of zinc in adolescent girls. The specific objectives of the study were:

- (1) To study zinc status and its association with the profile of other micronutrients, cognitive performance and taste acuity of the adolescent girls.
- (2) To examine the diet patterns of vegetarian adolescent girls for zinc adequacy and devise recipes to improve bio-available zinc intakes.
- (3) To develop and validate a nutrient quality index to assess micronutrient sufficiency of diets using statistical techniques and bioavailability of micro-
- (4) To examine efficacy of dietary supplementation on blood micronutrient levels and functional indices of zinc.

#### Material and methods

#### **Participants**

The study was conducted in two secondary schools from Pune City, Maharashtra State, in Western India, during 2005–2007. In all, 662 girls participated in the study. A physician conducted clinical examinations to assess health status of the girls.

### Exclusion criteria

Girls who were currently suffering or those who had suffered the previous fortnight from any illness such as fever, respiratory or gastro-intestinal infections or those undergoing medical treatment or taking multi-vitamin mineral supplements as per a physician's recommendation were excluded.

Thirty-two girls who met the exclusion criteria were excluded. Thus in all, 630 apparently healthy girls aged 10-16 years from the participating schools were recruited for the study.

Prior permission of the school authorities was taken to conduct the study in schools. Details of the procedures involved in the study were explained to the parents. After giving consent, girls were informed about the protocol of the study. The study protocol was reviewed and approved by the ethics committee of Zensar Foundation of India, Pune, under the chairmanship of a retired professor of the International Institute of Population Sciences, Mumbai. All of the parents gave their written informed consent, and the girls voluntarily participated in the study.

#### Outcome measures

Diet was assessed by 24-hour recall method on three random days including Sunday. Diet patterns were identified by principal component analysis. Nutrient intakes were estimated using cooked foods database (53) and nutritive value tables (54).

Venous blood samples (10 ml) were collected at 7.30 am after an overnight fast for each girl. Haemoglobin (Hb) was estimated using Automated Cell counter (ActDiff II, Beckman Coulter, Fullerton, CA, USA). Serum ferritin was estimated by Fully Automated Bidirectionally Interfaced Chemi Luminescent Immuno Assay. Plasma levels of zinc, vitamin C, β-carotene and retinol were measured as per the methods in the manual by National Institute of Nutrition, India (55), and erythrocyte zinc was measured as per the method described by Kenny et al. (56). Reference serum (RANDOX Laboratories, India) was used as a standard for blood estimations.

Cognitive performance was evaluated using a group of four tests standardised on Indian children; Simple Reaction Time (SRT), Recognition Reaction Time (RRT), Raven's Progressive Matrices (RPM) and visual Memory

on each participant (57–59). SRT and RRT measure the cognitive speed concerning perception, processing and motor response organisation for reaction tasks and visual search. SRT and RRT were measured using a visual reaction time apparatus (Anand Agencies, Pune, India) designed as per the procedure given by Woodworth et al. (57). RPM is a nonverbal test of performance that measures a person's ability to form perceptual relations and reason by analogy in research settings and form comparisons (58). A short-term visual memory test was carried out for measuring the ability of the girls to retain and reproduce information within a short time span. This test was adopted from the subtest of PGI memory scale developed by the Post Graduate Institute of Medical Education and Research, Chandigarh, India, for children and adolescents (59).

Taste acuity was assessed by identifying recognition threshold for salt (RTS) using 10 different salt solutions ranging from 0 to 25 mmol/l (40). More details of the measurements and methods used in the study are presented in five research papers (60-64).

#### Results and discussion

Average age, weight, height and body mass index of the girls were  $12.21 \pm 1.11$  years,  $33.05 \pm 7.22$  kg,  $143.74 \pm$ 8.24 cm and  $15.81 \pm 2.62$  kg/m<sup>2</sup>, respectively. Twentyfour percent of the girls were below the 5th percentile and 2.1% were above the 85th percentile of the Indian reference BMI for age (65).

Family size of the girls ranged from 2 to 11 persons in the household, with an average of  $5\pm2$  persons. Amongst fathers, 84.4% worked as salaried employees and the remaining were self-employed. Parents' educational status indicated that 13.8% of the fathers and 9.0% of the mothers completed high school, and 18.7% of the fathers and 13.2% of the mothers completed a college education.

# Nutrient profile and functional indices of zinc in adolescent girls

The mean intake of energy and protein was  $1373 \pm 381$ kcal/d and  $30.4 \pm 9.6$  g/d, respectively. Comparison with age-sex-specific reference intake indicated that these intakes were 68.7 and 60% of recommendation (24). Average calcium, iron, β-carotene and vitamin C intakes were  $289 \pm 146$ ,  $6.9 \pm 2.3$ ,  $619 \pm 564$  and  $25.6 \pm 14.5$  mg/d, respectively. Thus, average intakes of micronutrients were 30-60% of the respective RDI, indicating dietary deficiencies of these minerals and vitamins in the girls. The present study has offered estimates of prevalence of dietary micronutrient deficiencies in Indian urban adolescent girls, which are in agreement with previous findings on rural Indian adolescents (13, 16) and available studies on urban slum adolescents (14, 15). However, data on zinc intake are missing in these studies and the current literature. We have observed that average zinc intake  $(3.6 \pm 1.2 \text{ mg/d})$  of the girls was low, around 40% of the Indian RDI for zinc (66), with almost all girls having their zinc intake below RDI.

Plasma zinc levels were low (<0.7 mg/l) (41) in 72.4%, and erythrocyte zinc levels were low (<8 µg/g of packed cells) (56) in 23.6% of the girls. Prevalence of anemia (Hb < 12 g/dl) was 27.2%, and 26.6% girls had low serum ferritin lpar;<12 μg/l) (67). Plasma retinol was below the normal cutoff of 200 μg/l (68) in 65.4% girls. Plasma vitamin C level of 10.8% girls was below the normal cutoff of 0.2 mg/dl (69 p. 534), and 67.6% of the girls had low plasma vitamin C status. This high prevalence of micronutrient deficiencies confirmed inadequate dietary intakes of micronutrients by the girls from the study group.

Little information is available on the measurement of cognitive performance in adolescents. Paper I described the cognitive performance and assessed its relation with status of zinc and other micronutrients (60). The data on cognitive scores in our girls indicated that 60% of the girls were slow in simple reaction tasks, 71.5% of the girls had their RPM scores below 50th percentile and memory deficits were seen in 44% girls (60). Reaction time and RPM percentiles of majority of the girls were below the normal range, indicating deficits in the cognitive performance of the girls.

To explore the relative significance of dietary nutrient intakes with cognitive performance, weighted multiple regression adjusted for energy intake was carried out. The analysis revealed that intakes of β-carotene, iron and zinc were significant predictors for SRT (p < 0.01). Other cognitive scores did not exhibit significant association with micronutrient intakes. Further, our study also examines association of biomarkers of iron and zinc with cognitive scores of the girls. We have noted that plasma zinc and erythrocyte zinc were negatively correlated with SRT and RRT and positively with Memory and RPM (p < 0.05), whereas Hb was significantly correlated with RRT (p < 0.05) in girls with iron deficiency anaemia.

Taste RTS was found to be high in 45.2% girls, indicating impaired taste function. RTS showed significant negative correlation with erythrocyte zinc (r = -0.25, p < 0.05),

implying that lower the zinc status higher would be the salt concentration for taste perception.

Thus, our data indicated high prevalence of micronutrient deficiencies including zinc in adolescent girls. Moreover, we observed improvement in cognitive performance and taste perception with increased blood zinc levels in the girls. Therefore, an attempt has been made to develop zinc-rich recipes to improve total and bioavailable zinc intake of the girls.

# Diet patterns of adolescent girls and food-based approach for improving zinc intake

Description of diet patterns

In paper II (61), dietary food intakes on three random days by 24-hour recall were analysed for investigating prevailing diet patterns in the girls. It was observed that the girls were consuming rice-legumes meals, wheatlegumes/vegetables, mixed meals or bakery items. In all, the consumption of 86 different food items was reported by the girls during the 3 days' diet recall. Of these, 19 foods were different cereals preparations comprising rice, wheat or millets,; 16 were legume preparations; seven different bakery products; five leafy vegetables; 14 other vegetables; 13 fried foods; six fruits; and six milk and milk products. These foods were categorised into 12 food groups based on their major ingredients, such as rice, whole wheat, pearl millet, sorghum, bakery products, fried foods, sprouts, un-sprouted legumes, leafy vegetables, other vegetables, milk and milk products and fruits. For example, plain rice, rice flakes and puffed rice were categorised under the Rice food group, or foods such as unleavened pancakes (chapatti, paratha, roti) were categorised under the Wheat group.

Food intake data from 12 food subgroups were used to identify diet patterns of the girls. Principal component analysis was performed to identify diet patterns using orthogonal rotation method (varimax). Food subgroups with factor loading >0.3, eigenvalues and percentage of variance explained by each of the major dietary patterns identified in adolescent girls is given in Table 1. The factor loading indicated the degree to which the girls' diet conformed to one of the dietary patterns identified. Five patterns with eigenvalues greater than one

Table 1. Food subgroups, eigenvalues and percentage of variance explained by major dietary patterns identified adolescent girls

Diet pattern <sup>a</sup>	Food subgroups (factor loading <sup>b</sup> )	Eigenvalues	Variance explained (%)
Rice meal	Rice (0.794), un-sprouted legumes (0.768)	1.67	13.93
Snacks-type meal	Fried foods (0.781), Bakery products (0.336), fruits (0.764)	1.40	11.70
Wheat based meal	Whole wheat (0.457, milk (0.604), sprouts (0.300)	1.32	11.04
Pearl millet meal	Pearl millet (0.300), sprouts (0.535), leafy vegetable (0.444)	1.16	9.70
Sorghum meal	Sorghum (0.859), leafy vegetable (0.449)	1.06	8.83

<sup>&</sup>lt;sup>a</sup>Extraction method: Principle component analysis.

<sup>&</sup>lt;sup>b</sup>Varimax- rotated factor loading for the food subgroups.

were identified, which explained about 55.1% of the variance (Table 1). The patterns were characterised by typical combination of major cereal with food subgroups, namely (1) Rice meal, (2) Snack type meal, (3) Wheat meal, (4) Pearl millet meal and (5) Sorghum meal (61).

These five diet patterns were evaluated with respect to healthy food intakes using Mann-Whitney test. The results indicated that consumption of healthy foods mainly whole grains, sprouts and vegetables were high in Wheat, Pearl millet or Sorghum-based patterns than the Rice meal and the Snacks type meal (p < 0.05). In general, girls with the Rice meal and the Snacks type meal had low micronutrient intakes and low intake of bio-available zinc and iron than girls from other diet patterns (p < 0.05). However, all diet patterns were deficient in micronutrients including zinc when compared with the RDI (24, 66); mainly due to low intakes of healthy foods by the girls.

# Development of recipes to increase total and dialysable zinc content

Recipes were devised considering the dietary habits of the girls and representing each of the five diet patterns. Recipes were formulated (1) using foods that have high zinc contents such as pearl millet, chick peas, soybean, rajma, green gram, amaranthus seeds and onion stalks (54). Amongst the zinc-rich foods, selection was made on the basis of higher bio-availability (32) and local availability; (2) zinc density of the recipes was increased by using small amounts of sesame, amaranthus and pumpkin seeds; and (3) using methods like sprouting/fermentation, which increase the bio-availability of recipes.

The new zinc rich recipes were analysed in the laboratory for their nutrient content. Zinc content was determined by using atomic absorption spectrometer. In vitro zinc, dialysability of the recipes was determined by simulating gastrointestinal conditions and atomic absorption spectrometry (model 3110, Perkin Elmer, Norwalk, CT, USA). Contents of β-carotene and vitamin C were measured by spectrophotometry (Beckman Coulter, Inc. Brea, CA, USA) as described in NIN manual [55]. Energy, protein, calcium and iron contents were computed using the Indian national database of nutrient composition of raw foods (54) and adjusting for observed moisture contents of cooked recipes.

Average energy and protein content per 100 g of cooked weight of the recipes was 205 kcal and 2.5 mg, respectively. Average content of zinc was 1.5 mg/100 g of cooked weight, which was three times more than the observed average daily intake (0.47 mg/ 100 g cooked weight) of the girls. Average zinc dialysability of the recipes was 18.3%, which was observed to be higher than 11% dialysability of the diets consumed by the girls. Our recipes were prepared using vegetarian sources of zinc. The zinc-rich snacks containing meat developed for

Kenyan schoolchildren provided 2.89 mg/225 g of zinc (37), which was similar to the zinc content of proposed study (3.3 mg/225 g). Thus, consumption of these new recipes as a supplement to the existing diet has potential to improve zinc intake of the vegetarian girls.

Contents of iron, calcium, \( \beta-carotene and vitamin C were 6.2 g, 105 mg, 716 µg and 4.4 mg, respectively. Comparison of means by the Kruskal-Wallis test indicated that the mean content of micronutrients such as iron, β-carotene, vitamin C of new recipes were significantly higher than the observed intakes of the girls (p < 0.01). Therefore, modification in the existing diet using these recipes can increase micronutrient intakes of the girls.

# Development of a diet quality index with special reference to micronutrient adequacy for adolescent

For assessing dietary inadequacies of zinc and other micronutrients, especially in vegetarian populations, a simple tool such as micronutrient quality index is required to identify at-risk individuals. It is important to consider the content as well as bio-availability of micronutrients for evaluation of dietary adequacy. We have developed Adolescent Micronutrient Quality Index (AMQI) to classify an individual with nutritional deficiencies (Paper III) (62) using the Indian dietary guidelines (24) and the recent US dietary guidelines for choice of healthy foods and habits (23). Food intake data collected through 24-hour recall method for 3 days were utilised to develop the AMQI. Information about frequency of consuming sprouts, fermented foods and habit of taking tea with meals was utilised to judge bioavailable intakes. Efficacy of the AMQI was assessed by studying its associations with nutrient intakes, micronutrient adequacy ratio, body mass index and plasma biomarkers of β-carotene, vitamin C and zinc. The AMQI is divided into 13 components, where each component represents a specific grouping of foods. Seven components measured the degree of compliance, with recommendations for the major food groups such as cereals and millets, legumes, milk and milk products, vegetables, fruits, sugar and visible fats/oils. Six components relate to healthy eating and micronutrient bioavailability such as adherence to at least 50% consumption of whole grains and micronutrient-dense legumes (i.e. chick pea, moth beans, lentil), food variety (23, 26, 70, 71), use of practices enhancing bio-availability and component to evaluate excess fried food intake (71). Each component was scored from zero indicating lack of adherence to its maximum score for full adherence, with intermediate scores calculated to indicate the degree of adherence with dietary recommendations. The total score ranged from 0 (worst) to 100 (best).

The mean AMQI score was  $41.5 \pm 9.4$ , and 86.8% girls had the AMQI score lower than 50, indicating inadequate dietary nutrient intakes by the girls. The majority of the girls showed adherence to three of the healthy choice components, i.e. at least 50% grains as whole grains, low frequency of consuming fried foods and greater food variety. Adherence by the girls to the consumption of micronutrient dense pulses, sprouts, salad and fermented foods and not consuming tea with meals was poor.

Association of socio-demographic variables with AMOI was evaluated using the data on family size, mother's and father's education and occupation. In our data, mother's education and occupation were significantly associated with AMQI. A logistic regression analysis of the AMQI with socio-demographic factors revealed that age, mother's education and occupation were significantly and positively associated with the AMQI  $(R^2 = 0.07, p < 0.01)$ .

Partial correlations of the micronutrient intakes with the AMQI scores after adjusting for energy intake and socio-demographic factors were at the range of 0.4-0.5 and were statistically significant (p < 0.01), which demonstrated the concurrent validity of the new index. Higher AMQI scores were associated with higher concentrations of plasma vitamin C (r = 0.26),  $\beta$ -carotene (r = 0.34) and zinc (r = 0.12) after controlling for socio-demographic factors (p < 0.05). Thus, the AMQI provides an updated measure to evaluate micronutrient quality of diets of lacto-vegetarian Indian adolescent girls.

Our index is the first of its kind to consider bioavailability aspects of diet in the evaluation of diet quality. We anticipate that such concept of emphasising adequate contents and bio-availability of micronutrients while constructing the diet quality index can be widely applicable to different lacto-vegetarian population groups, although some of the components of the index may require modification with respect to nutrient requirements of different age-sex groups and food habits.

# Efficacy of dietary supplementation in improving blood micronutrient status and functional indices of zinc in adolescent girls

Dietary intervention strategies have been identified as the principal approach to tackle nutritional problems in the population. We have developed diet supplements in the form of zinc-rich recipes to improve intake of zinc and other micronutrients. An intervention trial was conducted to determine the efficacy of proposed recipes in improving zinc and other micronutrient status (Paper IV) (63) and functional indices of zinc (Paper V) (64). Effect of the diet supplementation was viewed in light of supplementing ayurvedic zinc (Jasad Bhasma) tablet.

Diet and Jasad supplementation both showed a significant increase in plasma zinc (9.9 and 61.3%, respectively) (p < 0.01) (63). Further, the diet supplement

showed significant increase in blood status of β-carotene and vitamin C as compared to control group (p < 0.01). Thus, devising such recipes would prove a better sustainable strategy for promoting micronutrient status of adolescents.

Our results demonstrated the beneficial effects of zinc supplementation on cognitive scores of the girls by reducing reaction time (6-13%) and improving in Memory (25%) and RPM scores (15%) than the control group (64). Median recognition threshold for salt taste reduced significantly from 5 to 2.5 mmol/l after both diet and zinc supplementation (p < 0.01); however, it remained same in the control group. This suggests a positive effect of supplementation on cognitive performance and taste acuity of the girls.

As observed in our study, decrease in reaction time has been reported by Pennland et al. (72) in school children. Furthermore, our study demonstrated that zinc supplementation improved memory and non-verbal intelligence in adolescent girls as well as established positive effect of zinc supplementation on taste perception. Thus, zinc supplementation through diet or ayurvedic Jasad tablet not only improves zinc status of the girls but also was a useful strategy for improving cognitive performance and taste acuity function in adolescent girls.

#### Conclusions

The present research provided first-hand estimates of micronutrient deficiencies in a cohort of urban Indian adolescent girls. Our data demonstrated that the diets of the girls were deficient in zinc and other micronutrients. Blood biomarkers of zinc and vitamin A were below the normal range in majority of the girls. Dietary zinc deficiency and low blood zinc status lead to poor cognition and taste perception amongst adolescent girls. Therefore, there is a concurrent requirement of a simple tool to identify dietary micronutrient deficiencies and a diet intervention strategy to improve zinc and other micronutrient status of the girls. The new diet quality index i.e. AMQI has successfully assessed micronutrient deficiency risk among study population and thus provided an updated measure to assess micronutrient quality of diets of lacto-vegetarian adolescent girls. The AMQI may be modified suitably for different age-sex groups considering their specific needs for assessing their micronutrient status.

An intervention trial on a group of 180 girls has demonstrated effectiveness of the proposed recipes in improving zinc status. Large-scale studies are needed to confirm its potential as a diet diversification strategy for vegetarian population. This study is the only one so far to demonstrate a positive effect of zinc supplementation in improving cognitive performance and taste acuity of adolescent girls. Effect of zinc supplementation on other aspects of health such as bone development, infections and obesity may be studied in adolescents.

Thus, the findings of the research demonstrate the necessity of adopting healthy micronutrient-rich diets for positive health building in adolescents. Further, from a global perspective, the methodologies and approaches used to assess dietary inadequacies in the form of AMQI and diet diversification strategies could be useful to explore and alleviate problem of micronutrient deficiencies in population.

# **Acknowledgements**

This work was carried out at the Biometry & Nutrition Group, Agharkar Research Institute (ARI), Pune. I would like to express my sincere and profound thanks to my guide Dr. (Mrs.) Shashi A Chiplonkar. I am grateful to Dr. Bhushan Patwardhan and Vaidya Dr. Milind Kulkarni for their valuable suggestions. I am thankful to Mr. R C Vaidya, Deputy Director, State Public Health Laboratory, Pune; Jnana Prabodhini's Institute of Psychology; Depart of Psychology, Garware College, Pune and authorities of both the schools for their support. Thanks are also due to the adolescent girls and their parents, who participated in the study.

## Conflict of interest and funding

The author has no conflicts of interest. This study was funded as a part of the project by Zensar Foundation, India.

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